Also known as

CIN Agar; *Yersinia* Selective Agar

Specification

Solid differential medium used for the selective isolation of *Yersinia spp.* from highly polluted samples, according to ISO 10273 standard.

Formula * in g/L

Special peptone	20.000	Magnesium sulfate	0.010
Yeast extract	2.000	Neutral red	0.030
Mannitol	20.000	Crystal violet	0.001
Sodium pyruvate	2.000	Agar	15.000
Sodium chloride	1.000		
Sodium deoxycholate	0.500		

Final pH 7,4 ±0,2 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 30,25 g in 500 mL of distilled water and bring to the boil. Sterilize in the autoclave at 121ºC for 15 minutes. Let it cool to 50-55ºC and, aseptically, add the content of a vial of CIN Yersinia Selective Supplement (Art. No. DSHB3109). Homogenize and pour into plates.

Description

Cefsulodin-Irgasan™-Novobiocin Agar CIN Agar was originally formulated by Schiemann (1979) for detection of *Yersinia enterocolitica*. He subsequently (1982) revised it by substituting sodium deoxycholate for bile salts and reducing the novobiocin content. It relies on the use of selective inhibitory components sodium deoxycholate, crystal violet, cefsulodin, Irgasan® and novobiocin. The basic principle involved is fermentation of mannitol with localised pH reduction which forms a red colony due to the neutral red and a zone of precipitation due to the deoxycholate.
The characteristic appearance of *Yersinia spp.* colonies after an incubation of 18-24 hours at 30ºC or 48 hours at 22ºC on CIN Agar in air, are round, pink, about 2 mm in diameter with a dark pink centre and surrounded with a precipitation zone. Confirmatory tests are required.
Typical colonies of *Yersinia enterocolítica* will develop as a red bull's-eye surrounded by a transparent border, but will vary considerably among serotypes in colony size, smoothness and the ratio of the border to centre diameter. Most other organisms that are capable of growing on this medium produce larger colonies (> 2 mm in diameter) with diffuse pinkish centres and opaque outer zones. Some strains of *Serratia*, *Citrobacter* and *Enterobacter* on CIN Agar may give a colonial morphology resembling *Yersinia enterocolitica*.
These organisms can be differentiated by simple biochemical tests.

Necessary supplements

Yersinia Selective Supplement (Art. No. DSHB3109))
Vial Contents:
Necessary amount for 500 mL of complete medium.

Cefsulodin	7,50 mg
Irgasan®	2,00 mg
Novobiocin	1,25 mg

Distilled water (Solvent)

Technique

At present no single isolation procedure is available for the recovery of all pathogenic strains of Yersinia enterocolitica. The isolation procedure used will depend on the bio/serogroups of Yersinia spp. sought and on the type of sample to be examined. The ISO method for the detection of presumptive pathogenic Yersinia enterocolitica includes the parallel use of two isolation procedures:

1. Enrichment in Peptone, Sorbitol and Bile Salts (PSB) Broth for 2-3 days at 22-25ºC with agitation or 5 days without agitation; plating on CIN Agar directly and after alkaline treatment and incubation for 24 hours at 30ºC.

2. Enrichment in ITC (Irgasan®-Ticarcillin-Chlorate) Broth for 2 days at 24ºC; plating on SSDC (Salmonella-Shigella-Deoxycholate-Calcium Chloride) Agar and incubation for 2 days at 30ºC.

Quality control

Incubation temperature: 30 ± 2 °C **Incubation time:** 24-48 h

Inoculum: Practical range 50- 100 CFU (Productivity) /10^4-10^6 CFU (Selectivity) according to Eu Ph. & ISO 11133:2014/Amd 1:2018

Microorganism	Growth	Remarks
Yersinia enterocolitica ATCC® 9610	Good	-
Escherichia coli ATCC® 25922	Partial inhibition	
Staphylococcus aureus ATCC® 25923	inhibited	

References

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· WEAGANT, S.D. & P. FENG (2001) Yersinia, in "Compendium of Methods for the Microbiological Examination of Foods". 4th ed. Downes & Ito (Eds.) APHA. Washington. DC. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).